

**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH**

SUMMARY OF TOXICOLOGY DATA

Fluazinam

**Chemical Code # 3898, Tolerance # 51977
SB 950 #**

5/17/11

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect indicated
Chronic toxicity, dog:	No data gap, no adverse effect indicated
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, no adverse effect indicated
Teratology, rat:	No data gap, possible adverse effect
Teratology, rabbit:	No data gap, no adverse effect indicated
Gene mutation:	No data gap, no adverse effect indicated
Chromosome effects:	No data gap, no adverse effect indicated
DNA damage:	No data gap, no adverse effect indicated
Neurotoxicity:	No data gap, possible adverse effect

Toxicology one-liners are attached.

All record numbers through 254039 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

indicates a study on file but not yet reviewed.

File name: T110517

Revised by T. Moore, 5/17/11

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

****0035, 245980;** "B-1216: Toxicity to Rats by Dietary Administration for 2 Years" (Chambers, P.R. et al., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, Study identifier ISK 43/920649, 06/14/1993). 870.4100. B-1216 Technical (Batch No. 8412-20, purity = 95.3%) was administered by admixture with the diet to 25 Crl:CD (SD) BR rats per sex per dose at dose levels of 0 (untreated diet), 25, 50, and 100 ppm (0, 1.0, 1.9, and 3.9 mg/kg/day, respectively for males and 0, 1.2, 2.4, and 4.9 mg/kg/day, respectively for females) for 104 weeks. Mortality totals were as follows- males: 17/25, 12/25, 18/25, 16/25, respectively; females: 7/25, 11/25, 7/25, 12/25, respectively. No treatment-related effect on body weight or food consumption was observed. Ophthalmoscopic examinations of the eyes of all animals in the control and high dose groups revealed no treatment-related ocular changes. A dose-related decrease in the mean hemoglobin level was observed in females at 100 ppm during weeks 26, 52, 78, and 104. Serum chemistry investigations revealed no treatment-related effects. Urinalysis revealed no treatment-related effects. Treatment-related increases in mean adjusted liver weight in both sexes and in mean adjusted testes/epididymides weight (body weight used as a covariate) at 100 ppm were observed. Macroscopic examination revealed treatment-related small and/or flaccid testes in males at 100 ppm. There was a treatment-related increase in neoplasm (pituitary adenocarcinoma) in females at 100 ppm. Microscopic examination revealed a treatment-related increase in the degree of tubular atrophy in the testes in males at 100 ppm; no other histopathological findings were determined to be treatment-related. **Possible adverse effect:** pituitary adenocarcinoma. NOEL (M) = 1.9 mg/kg/day (50 ppm), NOEL (F) = 2.4 mg/kg/day (50 ppm) based on increases in mean adjusted liver weight in both sexes and in mean adjusted testes/epididymides weight in males (body weight used as a covariate), pituitary adenocarcinoma (females), and small and/or flaccid testes and an increase in the degree of tubular atrophy in the testes (males). **Acceptable.** (Corlett and Leung, 09/07/2010)

****0044, 246002, 246003, 246004, 246005;** "B-1216: Potential Carcinogenicity and Chronic Toxicity Study in Dietary Administration to Rats for 104 Weeks" (Mayfield, R. et al., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, HRC Report No. ISK 8/87263, 08/25/1988). 870.4300. B-1216 (Batch No. 8412-20, purity = 95.2%) was administered by admixture with the diet to 50 Sprague-Dawley rats per sex per dose at dose levels of 0 (untreated diet), 1, 10, 100, and 1000 ppm (0, 0.04, 0.38, 3.82, and 40 mg/kg/day, respectively for males and 0, 0.05, 0.47, 4.87, and 53 mg/kg/day, respectively for females) for 104 weeks with 10 additional animals per sex per dose scheduled for interim sacrifice after 52 weeks of treatment. Mortality totals for the main group animals were as follows- males: 36/50, 32/50, 34/50, 28/50, 28/50, respectively; females: 32/50, 25/50, 26/50, 29/50, 20/50, respectively. For the interim group animals, 2 males at 100 ppm and 1 male at 1000 ppm were sacrificed or found dead prior to the scheduled sacrifice at week 53. Treatment-related straw discoloration of the fur in both sexes was observed at 1000 ppm. Treatment-related decreases in mean body weight and mean food consumption were observed in both sexes at 1000 ppm. Ophthalmoscopic examinations of the eyes of 10 male and 10 female animals in the control and high dose groups revealed no treatment-related ocular changes. A dose-related decrease in the mean red blood cell, hemoglobin, and hematocrit levels was observed in both sexes at 100 and 1000 ppm during the treatment period. A treatment-related increase in the mean cholesterol level was observed in both sexes at 100 and 1000 ppm. Urinalysis revealed no treatment-related effects. A treatment-related increase in mean adjusted liver weight in both sexes at 1000 (both interim and terminal sacrifice groups) was observed. Macroscopic examination revealed treatment-related pale liver in terminal group animals at scheduled sacrifice in both sexes at 1000 ppm. Micropathological examination revealed no treatment-related neoplastic tumors in the interim sacrifice animals or in the terminal sacrifice animals. Microscopic examination revealed treatment-related non-neoplastic findings in terminal group animals surviving to scheduled termination that included an increase in incidence and/or severity in alveolar epithelialization in the lungs in both sexes at 1000 ppm, sinus histiocytosis in the lymph nodes in females at 1000

ppm, eosinophilic hepatocytes in the liver in both sexes at 1000 ppm, centrilobular hepatocyte vacuolation and rarefaction in the liver in both sexes at 1000 ppm, bile duct hyperplasia in the liver in both sexes at 1000 ppm, pericholangitis in the liver in both sexes at 1000 ppm, centrilobular sinusoidal dilatation in the liver in both sexes at 100 and 1000 ppm, centrilobular fat in the liver in both sexes at 1000 ppm, exocrine atrophy in the pancreas in both sexes at 100 and 1000 ppm, acinar epithelial vacuolation in the pancreas in females at 1000 ppm, cortical hyperplasia and vacuolation in the adrenals in females at 1000 ppm, and testicular atrophy at 100 and 1000 ppm. **No adverse effects.** NOEL (M) = 0.38 mg/kg/day (10 ppm), NOEL (F) = 0.47 mg/kg/day (10 ppm) based on a microscopically observed increase in lesions in the liver and pancreas. **Acceptable.** (Corlett and Leung, 10/06/2010)

CHRONIC TOXICITY, RAT

See Combined, Rat above.

CHRONIC TOXICITY, DOG

****0034, 245978, 245979;** "B-1216: 52-Week Toxicity Study in Oral Administration to Beagle Dogs" (Broadmeadow, A., Life Science Research Limited, Eye, Suffolk, England, LSR Report No. 86/ISK055/512, 04/07/1987). 870.4100. B-1216 (Lot no. 8412-20, purity = 95.3%) was administered by gelatin capsule to 6 beagle dogs per sex per dose at dose levels of 0 (one empty gelatin capsule), 1, 10, and 50 mg/kg/day once a day 7 days a week for at least 52 weeks. No animals died during the treatment interval. Treatment-related incidences of salivation (at 50 mg/kg/day) and nasal dryness (at 10 and 50 mg/kg/day) in both sexes were observed. A treatment-related decrease in body weight was observed in females at 50 mg/kg/day over the 52-week treatment interval. Treatment-related decreases in mean red blood cell, hemoglobin, and hematocrit levels were observed in both sexes at 50 mg/kg/day; a treatment-related increase in the mean white blood cell level was observed in both sexes at 50 mg/kg/day. A treatment-related increase in the mean alkaline phosphatase level was observed in both sexes at 50 mg/kg/day; a treatment-related increase in the mean cholesterol level in males was observed at 50 mg/kg/day. A treatment-related increase in mean relative liver weight in both sexes at 50 mg/kg/day was observed. Necropsy revealed abnormal contents throughout the gastrointestinal tract in both sexes at 50 mg/kg/day and, in particular, abnormal contents in the jejunum of both sexes at 10 and 50 mg/kg/day. Microscopic examination revealed a treatment-related increase in the degree of mucosal lymphoid hyperplasia in the stomach in males at 10 and 50 mg/kg/day, a treatment-related increase in incidence and/or degree of vacuolation of white matter in the cerebrum and in the cerebellum/pons/medulla/ midbrain in both sexes at 50 mg/kg/day, and an increase in vacuolation of white matter in the spinal cord in females at 50 mg/kg/day. No histopathological lesion was observed in the tapetal or non-tapetal retina of any control or treated animal. **No adverse effects.** NOEL (M/F) = 1 mg/kg/day based on abnormal contents in the jejunum (both sexes), an increase in degree of mucosal lymphoid hyperplasia in the stomach (males), and increased incidences of nasal dryness (both sexes). **Acceptable.** (Corlett and Leung, 08/25/2010)

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

****0036, 245981, 245982, 245983** (re-examination of the nervous tissue); "B-1216: Potential Carcinogenicity Toxicity Study in Dietary Administration to Mice for 104 Weeks" (Mayfield, R. et al., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, HRC Report No. ISK 8/87264, 09/29/1988). 870.4200. B-1216 (Batch No. 8412-20, purity = 95.3%) was administered by admixture with the diet to 52 CD-1 mice per sex per dose at dose levels of 0 (untreated diet, 2 separate control groups of 52 animals per sex), 1, 10, 100, and 1000 ppm (0, 0, 0.12, 1.12, 10.72, and 107 mg/kg/day, respectively for males and 0, 0, 0.11, 1.16, 11.72, and 117 mg/kg/day, respectively for females) for 104 weeks. Mortality totals were as follows- males: 33/52, 31/52, 35/52, 29/52, 32/52, 29/52, respectively; females: 17/52, 26/52, 21/52, 19/52, 19/52, 25/52, respectively. No treatment-related clinical signs were reported. No treatment-related effects on body weight or food consumption were observed. Hematology investigations revealed

no treatment-related effects. A treatment-related increase in mean adjusted liver weight in females at 100 ppm and in both sexes at 1000 ppm was observed. Macroscopic examination revealed a treatment-related increase in the number of masses in the liver, pitted liver or liver with irregular surface, pale liver or liver with pale areas, and liver with accentuated lobular markings in males at 1000 ppm, and pitted liver or liver with irregular surface, pale areas on the liver, and an increase in the number of uterine masses in females at 1000 ppm. Microscopic examination revealed treatment-related liver tumors (benign and/or malignant) in males at 1000 ppm. Microscopic examination revealed treatment-related non-neoplastic findings that included an increase in incidence of areas/foci of basophilic and/or eosinophilic hepatocytes (some vacuolated) in males at 1000 ppm, minimal to moderate granulomatous hepatitis in the liver of males at 100 and 1000 ppm and in females at 1000 ppm, and brown pigmented macrophages in the liver in males at 100 and 1000 ppm and in females at 1000 ppm. Microscopic examination of the brain and spinal cord revealed a treatment-related increase in incidence and severity of vacuolation of the white matter in the cerebrum and cerebellum/pons/medulla and an increase in incidence of vacuolation of white matter in the spinal cord in males at 1000 ppm and an increase in severity of vacuolation of white matter in the cerebrum and cerebellum/pons/medulla in females at 1000 ppm. **Possible adverse effect:** treatment-related liver tumors NOEL (M) = 1.12 mg/kg/day (10 ppm), NOEL (F) = 1.16 mg/kg/day (10 ppm) based on an increase in mean adjusted liver weight and a microscopically observed increase in lesions in the liver. **Acceptable.** (Corlett and Leung, 10/29/2010)

****0037, 245984;** "Technical Fluazinam: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice (Vols. 1-13) Contains: Final Report and Addendums 1 & 2" (Chambers, P. R., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Document No. ISK 50/950671, 12/19/1996). 870.4200. Technical Fluazinam (Batch No. Lot 1030/91, purity = 97.0%) was administered by admixture with the diet to 50 CrI: CD-1 (ICR) BR mice per sex per dose at dose levels of 0 (untreated basal diet), 1000, 3000, or 7000 ppm (0, 126, 377, and 964 mg/kg/day, respectively for males and 0, 162, 453, and 1185 mg/kg/day, respectively for females) for 97 weeks for females and 104 weeks for males with 20 additional animals per sex at 0 and 7000 ppm (satellite group animals) for 78 weeks. Mortality totals (main group animals) were as follows- males: 32/50, 24/50, 29/50, 34/50, respectively; females: 21/50, 24/50, 23/50, 37/50, respectively. Treatment-related periorbital hair loss was observed in females at 3000 ppm and in both sexes at 7000 ppm. A treatment-related decrease in body weight gain was observed in males at 7000 ppm from week 4 to week 36. No treatment-related effect on food consumption in males and females was observed. Hematology investigations revealed no treatment-related effects. A treatment-related increase in mean adjusted liver weight at all treatment levels in both sexes in both the main and satellite groups and a treated-related increase in mean adjusted brain weight in males at 7000 ppm were observed. Macroscopic examination of the interim sacrifice group animals revealed treatment-related changes in the liver in both sexes including enlargement, paleness, and accentuated lobular markings. Macroscopic examination of the main group animals revealed treatment-related changes in the liver including accentuated lobular markings in both sexes at all treatment levels, enlargement, pale areas, dark areas, and brown color in males at all dose levels, and enlargement and pale areas in females at 3000 and 7000 ppm. Microscopic examination on the interim sacrifice group animals revealed no treatment-related neoplastic findings. Microscopic examination on the main group animals revealed treatment-related liver tumors (benign and/or malignant) in males at 3000 and 7000 ppm. Microscopic examination on the interim group animals revealed treatment-related non-neoplastic findings in the liver that included an increase in incidence of basophilic and eosinophilic vacuolated hepatocytes, centrilobular and midzonal hepatocyte enlargement, centrilobular and midzonal hepatocytes with pale/vacuolated cytoplasm, and aggregates of macrophages containing brown pigment (mainly centrilobular) in both sexes. Microscopic examination on the main group animals revealed the same treatment-related non-neoplastic findings in the liver that are listed for the interim group animals above in both sexes with some of the findings present at all treatment levels. Microscopic examination of the brain and spinal cord of the interim group animals revealed a treatment-related vacuolation of the white matter in both sexes; microscopic examination of the brain and spinal cord of the main group animals revealed a treatment-related increase in incidence of vacuolation of white matter in both the spinal cord and brain in both

sexes at 3000 and 7000 ppm with widespread vacuolation in both the spinal cord and brain in both sexes at 7000 ppm. The central nervous system and liver were identified as target organs. **Possible adverse effect:** treatment-related liver tumors. NOEL not determined; treatment related effects were observed at all treatment levels. **Acceptable.** (Corlett and Leung, 11/22/2010)

0037, 245985; "Addendum Report To: Technical Fluazinam: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice (Vols. 1-13) (Contains Report and Addendums 1 & 2)" (Chambers, P. R., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Document No. ISK 50/950671, 12/19/1996). The author of a study previously reviewed (Document No. 51977-0037, Record No. 245984) states in this addendum to that study that a further peer review of a small number of slides from male mice showing hepatocellular tumors and other lesions reported as liver masses was undertaken and, in light of this later review, the diagnosis of three animals was changed (page 6). In one control male mouse, an area originally characterized as basophilic and vacuolated hepatocytes was re-characterized as a hepatocellular adenoma. In another control male mouse, an area (one of several) originally characterized as eosinophilic hepatocytes was re-characterized as a hepatocellular adenoma. In a mouse at 1000 ppm, a tumor originally characterized as a hepatocellular adenoma, was re-characterized as a hepatocellular carcinoma. **Supplemental data.** (Corlett and Leung, 11/23/2010).

0038, 245988; "Pathology Working Group (PWG) Report on Liver Tumours in Study No. ISK 50/950671- Technical Fluazinam: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice" (Gopinath, C., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Document No. ISK 50/950671 PWG, 08/24/2000). The male liver tumor data originally examined in a oncogenicity study (Document No. 51977-0037, Record No. 245984) and later peer reviewed again (Document No. 51977-0037, Record No. 245985) was examined again by a Pathology Working Group (PWG) consisting of three independent Consultant Pathologists. In case of the control male mouse where an area originally characterized as basophilic and vacuolated hepatocytes and was re-characterized as a hepatocellular adenoma, the PWG determined that the lesion should be characterized as eosinophilic and vacuolated hepatocytes. In the mouse at 1000 ppm where a tumor was originally characterized as a hepatocellular adenoma and was re-characterized as a hepatocellular carcinoma, the PWG determined that the lesion should be characterized as hepatocellular adenoma. **Supplemental data.** (Corlett and Leung, 11/23/2010).

REPRODUCTION, RAT

** 0041, 245997; "B-1216: Effects Upon Reproductive Performance of Rats Treated Continuously Throughout Two Successive Generations" (Tesh, J.M. et al., Life Science Research, Eye, Suffolk, England, LSR Report No. 87/ISK068/097, 12/14/1987). 870.3800. B-1216 (Lot No. 8412-20, purity = 95.3%) was administered in the diet to 24 males and 24 female CD (Sprague-Dawley origin) rats per sex per dose for 2 generations at dose levels of 0 (basal diet only), 20, 100, and 500 ppm (0, 1.05-2.40, 5.22-12.0, and 26.2-59.6 mg/kg/day, respectively, for F0 males prior to mating, 0, 1.43-2.35, 6.74-11.9, and 33.6-58.8 mg/kg/day, respectively, for F0 females prior to mating, 0, 1.17-3.46, 5.82-17.7, and 29.6-87.7 mg/kg/day, respectively, for F1 males prior to mating, 1.55-3.61, 7.48-17.6, and 40.0-88.2 mg/kg/day, respectively, for F1 females prior to mating). The premating period was 11 weeks from the start of the study for the F0 parents and 11 weeks after weaning for the F1 generation parents. Treatment continued through mating, gestation, and lactation for both generations. The litters were weaned at day 21 *post partum*. No treatment-related mortalities occurred in the adult F0 and F1 generation animals. A treatment-related decrease in mean body weight gain was observed in both sexes in the premating F1 parents at 500 ppm; a treatment-related decrease in mean body weight was observed in F1 females during gestation at 100 and 500 ppm. A treatment-related decrease in mean live litter size of F1 females was observed at 500 ppm. A treatment-related decrease in both F1 and F2 mean pup weight on day 21 of lactation at 500 ppm was observed. Macroscopic and microscopic examination of the F0 and F1 parents (males and females) and offspring revealed no toxicologically significant abnormalities. **No adverse effects. Parental NOEL (M)** (prior to mating) = 5.82-17.7 mg/kg/day (100 ppm), based on decreased body weight gain of F1 males,

Parental NOEL (F) = 1.55-3.61 mg/kg/day (20 ppm), based on decreased body weight gain of F1 females during gestation; **Reproductive NOEL** = 20 ppm, based on a decrease in mean live litter size of F1 females; **Offspring NOEL** = 100 ppm based on a decrease in mean pup weight. **Acceptable.** (Corlett and Leung, 8/06/2010)

0042; 246000; "Fluazinam Technical (B1216): Effects Upon Reproductive Function and Performance in Rats, 1. Dose Range Finding Study" (Tesh, J.M. et al., Life Science Research, Eye, Suffolk, England, Study No. (referred to as Document No.) 84/ISK043/547, 01/28/1985). B-1216 (Batch No. 8303-2, purity = 98.5%) was administered in the diet to 10 CD (Sprague-Dawley origin) rats per sex per dose at dose levels 0 (basal diet only), 5, 20, 100, or 1000 ppm (0, 0.29-0.35, 1.13-1.45, 5.54-6.94, and 55.0-66.0 mg/kg/day, respectively, for males prior to mating, 0, 0.36-0.41, 1.40-1.58, 7.06-8.01, and 68.7-73.0 mg/kg/day, respectively, for females prior to mating) starting 29 days before pairing and ending after the successful littering by all the females (males) or until termination at day 21 *post partum* (females). Each female was cohabited with one male until evidence of mating was detected. No parental mortalities occurred. A treatment-related decrease in mean body weight was observed in females at 1000 ppm prior to mating; no treatment-related effect on body weight was observed in males. A treatment-related decrease in mean food consumption was observed in females at 1000 ppm prior to mating; no treatment-related effect of food consumption was observed in males. No mating and fertility effects were observed in treated animals when compared to the control animals. A treatment-related decrease in mean body weight was observed in F0 females at 1000 ppm during gestation and at the beginning of lactation. A treatment-related decrease in litter size was observed at 1000 ppm. A treatment-related increase in mean relative liver weight was observed in F0 and F1 generation males at 1000 ppm and in F0 and F1 generation females at 100 and 1000 ppm. Microscopic examination of the liver of the F0 animals revealed treatment-related increased periportal basophilia in males at 1000 ppm and in females at 100 and 1000 ppm and periportal fatty vacuolation in males at 1000 ppm; microscopic examination of the liver of the F1 animals revealed no treatment-related effects. **Parental NOEL (M)** (prior to mating) = 5.54-6.94 mg/kg/day (100 ppm) and **Parental NOEL (F)** (prior to mating) = 1.40-1.58 mg/kg/day (20 ppm), both based on increased mean relative liver weights and microscopic changes in the liver; **Offspring NOEL (M)** = 100 ppm and **Offspring NOEL (F)** = 20 ppm both based on increased mean relative liver weights. **Supplemental study** (because only 10 animals per sex per dose group were used and because the study was ceased after one generation). (Corlett and Leung, 07/22/2010)

0043; 246001; "B-1216: Effects Upon Reproductive Function and Performance in Rats, Second Dose Range-Finding Study" (Tesh, J.M. et al., Life Science Research, Eye, Suffolk, England, LSR Report No. 85/ISK050/295, 06/03/1986). B-1216 (Batch No. 8303-2, purity = 98.5%) was administered in the diet to 10 CD (Sprague-Dawley origin) rats per sex per dose at dose levels 0 (basal diet only), 20, 100, 250, or 500 ppm (0, 0.99-2.09, 4.92-10.3, 12.2-25.5, and 24.9-53.0 mg/kg/day, respectively, for males prior to mating, 0, 1.21-2.39, 6.05-11.2, 14.7-30.2, and 33.1-60.2 mg/kg/day, respectively, for females prior to mating) starting 85 days before pairing and ending after the successful littering by all the females (males) or until termination at day 21 *post partum* (females). Each female was cohabited with one male until evidence of mating was detected. No parental mortalities occurred. A treatment-related decrease in mean body weight gain was observed in males at 500 ppm from weeks 0-6 prior to mating and in females at 500 ppm from weeks 0-12 prior to mating; no effect on food consumption was observed. No effect on the body weight of the offspring was observed. No mating and fertility effects were observed in treated animals when compared to the control animals. A treatment-related increase in mean relative liver weight was observed in parental males at 250 and 500 ppm and in parental females at 500 ppm; no treatment-related effect on liver weight of the offspring was observed. Microscopic examination of the liver of the parental and offspring animals revealed no treatment-related effects. **Parental NOEL (M)** (prior to mating) = 4.90-10.3 mg/kg/day (100 ppm) and **Parental NOEL (F)** (prior to mating) = 14.7-30.2 mg/kg/day (500 ppm), based on increased mean relative liver weights; **Offspring NOEL (M/F)** = 500 ppm based on no effects at the highest dose tested. **Supplemental study** (because only 10 animals per sex per dose group were used and because the study was ceased after one generation). (Corlett and Leung, 07/27/2010)

TERATOLOGY, RAT

****0040, 245995;** "B-1216: Teratology Study in the Rat, Amended Final Report" (Willoughby, C.R. et al., Life Science Research Limited, Eye, Suffolk, England, Amended Final Report No. 91/ISK047/0820, 11/07/1991). 870.3700. B-1216 (Lot No. 8303-2, purity = 98.5%), prepared in corn oil, was administered as a daily dose by gavage to 20 mated female CD (Sprague Dawley origin) rats per dose at dose levels 0 (vehicle only), 10, 50, or 250 mg/kg/day on days 6 through 15 (inclusive) of gestation. No maternal deaths were observed. No treatment-related clinical signs were observed. A treatment-related decrease in maternal mean body weight gain was observed at 250 mg/kg/day during the day 6 to day 15 interval and a treatment-related decrease in maternal mean food consumption was observed at 250 mg/kg/day during the day 6 to day 8 interval. Macroscopic examination of the dams revealed no treatment-related changes. No treatment-related effects on fetus viability and posimplantation loss were observed. Treatment-related decreases in placental weights and fetal body weights were observed at 250 mg/kg/day. Treatment-related incidences of cleft palate/incomplete ossification or absence of palatine bones in fetuses at 250 mg/kg/day were observed. **Possible adverse effect:** Fetuses with cleft palate/incomplete ossification or absence of palatine bones. **Maternal NOEL** = 50 mg/kg/day (based on decreased mean weight gain and mean food consumption), **Developmental NOEL** = 50 mg/kg/day (based on decreased mean fetal weight and fetuses with cleft palate/incomplete ossification or absence of palatine bones). **Acceptable.** (Corlett and Leung, 05/19/2010)

Range-finding Teratology Study

0040, 245996; "B-1216: Effects of Oral Administration Upon Pregnancy in the Rat 1. Dosage Range-Finding Study" (Tesh, J.M. et al., Life Science Research, Eye, Suffolk, England, LSR Report No. 84/ISK042/314, 10/31/1984). B-1216 (Lot No. 8303-2, purity = 98.5%), prepared in corn oil, was administered as a daily dose by gavage to 7 mated female CD (Sprague-Dawley origin) rats per dose at dose levels 0 (vehicle only), 1, 10, 100, or 1000 mg/kg/day on days 6 through 15 (inclusive) of gestation. All maternal dams at 1000 mg/kg/day died or were sacrificed *in extremis*; no other mortalities occurred. Treatment-related clinical signs observed in dams at 1000 mg/kg/day included stained and ungroomed coat, lethargy, hunched posture, ataxia, flaccid muscles, and salivation; no treatment-related clinical signs were observed at the other dose levels. Treatment-related body weight loss was observed in dams at 1000 mg/kg/day; no treatment-related effect on body weight was observed at the other dose levels. No treatment-related effects were observed with respect to the number of implantations, the number of viable young or resorptions, and the number of pre- and post-implantation losses. No fetal malformations were observed. **Maternal NOEL** = 100 mg/kg/day (based on clinical signs, unscheduled deaths, and a decrease in body weight); **Developmental NOEL** = 100 mg/kg/day (based on no fetal malformations). **Supplemental Study** (only 7 impregnated females per dose level were used in the study). (Corlett and Leung, 07/07/2010)

TERATOLOGY, RABBIT

****0039, 245990;** "B-1216: Teratology Study in the Rabbit, Amended Final Report" (Tesh, J.M. et al., Life Science Research Limited, Eye, Suffolk, England, Amended Final Report No. 91/ISK069/0835, 11/07/1991). 870.3700. B-1216 (Lot No. 8412-20, purity = 95.3%), formulated in aqueous 1% w/v methylcellulose mucilage, was administered as a daily dose by gavage to 16-18 inseminated female New Zealand White rabbits per dose at dose levels 0 (vehicle only), 2, 4, 7, or 12 mg/kg/day on days 6 through 19 (inclusive) of gestation. Maternal deaths occurred as follows- 2/18, 1/16, 2/17, 3/17, and 2/16, respectively. A treatment-related decrease in maternal mean body weight gain was observed at 4 mg/kg/day and above during the day 6 to day 20 interval and a treatment-related decrease in maternal mean daily food consumption was observed at 7 and 12 mg/kg/day during the day 13 to day 19 interval. Microscopic examination of the does revealed treatment-related pancreatic hypertrophy in the liver and alveolar hemorrhage in the lungs at 4 mg/kg/day and above. A treatment-related increase in mean postimplantation loss was observed at 12 mg/kg/day. No treatment-related fetal abnormalities were observed. **No adverse**

effects. **Maternal NOEL** = 2 mg/kg/day (based on pancreatic hypertrophy in the liver), **Developmental NOEL** = 12 mg/kg/day (based on no effects at the highest dose tested). **Acceptable.** (Corlett and Leung, 06/22/2010)

**0039, 245991; "B-1216: Teratology Study in the Rabbit, Amended Final Report" (Tesh, J.M. et al., Life Science Research Limited, Eye, Suffolk, England, Amended Final Report No. 91/ISK049/0826, 11/07/1991). 870.3700. B-1216 (Lot No. 8303-2, purity = 98.5%), formulated in 1.0% w/v aqueous methylcellulose mucilage, was administered as a daily dose by gavage to 24 (control group) or 20 (each treated group) inseminated female New Zealand White rabbits per dose at dose levels of 0 (vehicle only), 0.3, 1.0, or 3.0 mg/kg/day on days 6 through 19 (inclusive) of gestation. Maternal deaths occurred as follows- 4/24, 0/20, 1/20, and 1/20, respectively.

No treatment-related effects on maternal mean body weight gain were observed. No treatment-related effects were observed in the maternal animals. No treatment-related fetal effects were observed. **No adverse effects.** **Maternal NOEL** = 3.0 mg/kg/day (based on no effects at the highest dose tested), **Developmental NOEL** = 3.0 mg/kg/day (based on no effects at the highest dose tested). **Acceptable.** (Corlett and Leung, 06/28/2010)

Range-finding Teratology Studies

0039, 245993; "B-1216: Tolerance Study in the Rabbit" (Tesh, J.M. et al., Life Science Research, Elm Farm Laboratories, Eye, Suffolk, England, LSR Report No. 84/ISK044/296, 11/02/1984). B-1216 (Batch No. 8303-2, purity = 98.5%) was formulated freshly each day in 1.0% w/v methyl cellulose and administered orally daily by gavage for 14 days to 2 female New Zealand White rabbits per dose at dose levels of 10 and 100 mg/kg/day. One animal at 100 mg/kg/day was found dead and the other was sacrificed *in extremis* on day 7. The two animals at 10 mg/kg/day were sacrificed on day 21. Treatment-related decreased body weight and reduced food intake and fecal output were observed in all animals. Necropsy on the animals at 10 mg/kg/day revealed no abnormalities in one animal and respiratory and gastrointestinal disorders and a yellow liver in the other animal; necropsy on the animals at 100 mg/kg/day revealed signs of autolysis in the animal found dead and signs of gastrointestinal tract disorder in the animal sacrificed *in extremis*. **No adverse effects indicated.** NOEL not determined. **Supplemental study** (non-guideline study: only 2 females per dose level were used and only two dose levels were used). (Corlett and Leung, 06/29/2010)

0039, 245994; "B-1216: Effects of Oral Administration Upon Pregnancy in the Rabbit 1. Dosage Range-Finding Study" (Tesh, J.M. et al., Life Science Research, Elm Farm Laboratories, Eye, Suffolk, England, LSR Report No. 84/ISK041/369, 11/19/1984). B-1216 (Lot No. 8303-2, purity = 98.5%), formulated in 1.0% w/v aqueous methyl cellulose mucilage, was administered as a daily dose by oral gavage to 5 inseminated female New Zealand White rabbits per dose at dose levels of 0 (vehicle only), 0.5, 2.5, 5.0, or 10.0 mg/kg/day on days 6 through 19 (inclusive) of gestation. Treatment-related maternal deaths occurred as follows- 0/5, 0/5, 0/5, 0/5, and 2/5 (both sacrificed *in extremis*), respectively. A treatment-related decrease in mean food consumption was observed in the maternal animals. Necropsy on the maternal does revealed pale areas on the left liver lobe in one animal at 5.0 mg/kg/day and accentuation of lobular pattern on liver in another animal at 5.0 mg/kg/day, and the liver pale and hardened or firm in 2 animals at 10.0 mg/kg/day. A treatment-related decrease in mean fetal weight was observed at 10.0 mg/kg/day. No treatment-related fetal malformations were observed. **No adverse effects.** **Maternal NOEL** = 2.5 mg/kg/day (based on decreased food consumption and macroscopic liver changes), **Developmental NOEL** = 5.0 mg/kg/day (based on decreased mean fetal weight). **Supplemental Study** (only 5 impregnated females per dose level were used in the study). (Corlett and Leung, 07/01/2010)

GENE MUTATION

0045, 246006; "Bacterial Reverse Mutation Test of Fluazinam Technical" (Ohtsuka, M. and Yamamoto, T., Hita Research Laboratories, Chemical Biotesting Center, Chemicals Inspection

and Medical Testing Institute, Hita, Japan, Test Code: K01-0560, 01/12/1989). 870.5100.

Duplicate cultures of *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and of *Escherichia coli* WP2 *uvrA* were exposed (direct plate incorporation method) to Fluazinam technical, Lot No. 109, purity = 95.3%, in the presence and absence of S9 rat liver fraction, at 0 (DMSO), 0.0313, 0.0625, 0.125, 0.25, 0.5, and 1 ug/plate (*S. typhimurium* strains without metabolic activation), at 0 (DMSO), 3.13, 6.25, 12.5, 25, 50, and 100 ug/plate (*S. typhimurium* strains with metabolic activation), and at 0 (DMSO), 15.6, 31.3, 62.5, 125, 250, and 500 ug/plate (*E. coli* strain, with and without metabolic activation) and incubated for 48 hours at $37 \pm 1^\circ\text{C}$. Positive controls were functional. There was no treatment-related increase in mutation frequency. **No adverse effects indicated. Unacceptable but possibly upgradable** with the submission of a scientific justification why 2 and not 3 plates per dose level were used in this study. (Corlett and Leung, 12/01/2010)

0045, 246007; "Bacterial Reverse Mutation Test of Fluazinam Technical" (Ohtsuka, M. and Yamamoto, T., Hita Research Laboratories, Chemical Biotesting Center, Chemicals Inspection and Medical Testing Institute, Hita, Japan, Test Code: K01-0561, 11/28/1988). 870.5100.

Duplicate cultures of *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and of *Escherichia coli* WP2 *uvrA* were exposed (direct plate incorporation method) to Fluazinam technical, Lot No. 8412-20, purity = 95.3%, in the presence and absence of S9 rat liver fraction, at 0 (DMSO), 0.0625, 0.125, 0.25, 0.5, 1, and 2 ug/plate (*S. typhimurium* strains without metabolic activation), at 0 (DMSO), 3.13, 6.25, 12.5, 25, 50, and 100 ug/plate (*S. typhimurium* strains with metabolic activation), and at 0 (DMSO), 15.6, 31.3, 62.5, 125, 250, and 500 ug/plate (*E. coli* strain, with and without metabolic activation) and incubated for 48 hours at $37 \pm 1^\circ\text{C}$. Positive controls were functional. There was no treatment-related increase in mutation frequency. **No adverse effects indicated. Unacceptable but possibly upgradable** with the submission of a scientific justification why 2 and not 3 plates per dose level were used in this study. (Corlett and Leung, 12/02/2010)

0045, 246010; "L5178Y/TK+/- Mouse Lymphoma Mutagenicity Test" (Dollenmeier, P., Ciba-Geigy Limited, Experimental Pathology, Tissue Culture Laboratories, Basel Switzerland, Test No. 840403, 07/31/1986). 870.5300. Cultures of the TK^{+/-} mouse lymphoma L5178Y cell line were treated with CGA 143 268 techn. (Batch No. KGL 3147/5, ES 4390, purity $\geq 95\%$), in the presence and absence of rat liver S-9 activation system, for 4 hours at concentrations of 0, 0.3, 0.6, 1.2, 1.8, 2.4, 2.7, and 3.0 ug/ml in the first experiment and 0, 0.5, 1.0, 2.0, 3.0, 4.0, 4.5, and 5.0 ug/ml in the second experiment. Positive controls of ethylmethane-sulphonate (EMS) (non-activated) and dimethylnitrosamine (DMN) (activated) were used. It was reported that no evidence of mutagenic effects was observed in this mammalian forward mutation system. **No adverse effects indicated. Unacceptable but possibly upgradable** with the submission of a detailed explanation on how the relative suspension growth, the relative cloning efficiency, and the mutant efficiency data were calculated. (Corlett and Leung, 12/15/2010)

**0045, 246012; "IKF-1216 Mammalian Cell Mutation Assay" (Ransome, S., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Document No. RIA 017/004090, 10/30/2000). 870.5300. Duplicate cultures of the TK^{+/-} mouse lymphoma L5178Y cell line were treated with IKF-1216 (Lot No. 109, purity = 95.3%), in the presence and absence of S-9 rat liver fraction, for 3 hours (24 hours in the second experiment for the without metabolic activation group) at concentrations ranging from 0.05 to 5.0 ug/ml (without metabolic activation) and from 0.5 to 20.0 ug/ml (with metabolic activation) in the first experiment and from 0.005 to 0.500 ug/ml (without metabolic activation) and from 0.5 to 10.0 ug/ml (with metabolic activation) in the second experiment. Positive controls were functional. Marked cell toxicity occurred in cultures with concentrations of the test article greater than 0.200 ug/ml without metabolic activation and greater than 9.0 ug/ml with metabolic activation. No increase in mutant frequency in the presence or absence of S-9 was observed where there was sufficient cell viability. Under the conditions

employed in this study, the test article was not found to be mutagenic where sufficient cell viability occurred. **No adverse effects indicated. Acceptable.** (Corlett and Leung, 12/09/2010)

CHROMOSOME EFFECTS

0045, 246013; "Chromosomal Aberration Test of Fluazinam Technical Using Cultured Mammalian Cells" (Kajiwara, Y. and Yamamoto, Hita Research Laboratories, Chemical Biotesting Center, Chemicals Inspection & Testing Institute, Hita, Japan, Document No. T-1663E, Test Code No. K06-0064, 09/30/1988). 870.5375. Duplicate cultures of Chinese hamster lung fibroblasts were treated with Fluazinam technical (Lot No. 109, purity = 95.3%) for 24 and 48 hours (in the absence of metabolic activation) and for 6 hours (in the presence of metabolic activation) at concentrations of 0, 1, 2, and 4 ug/ml (no metabolic activation) and 0, 2.375, 4.75, and 9.5 ug/ml (metabolic activation). Dividing cells were arrested at metaphase by treatment with colcemid 2 hours prior to the end of the incubation of the cells. Positive controls were functional. No treatment-related chromosomal aberrations were observed. **No adverse effects indicated. Acceptable. (Corlett and Leung, 12/21/2010)

DNA DAMAGE

0045, 246014; "IKF-1216 Technical: Micronucleus Test in Mice" (Matsumoto, K., Mitsukaido Laboratories, The Institute of Environmental Toxicology, Ibaraki, Japan, Study No. IET 98-0139, 03/08/1999). 870.5395. IKF-1216 Technical (Lot No. 8412-20, purity = 95.6%) was suspended in olive oil and administered by gavage to groups of ICR (Crj:CD-1) mice in the following manner: first, in a time course micronucleus study, 5 animals per sex received a 2000 mg/kg dose of the dosing material and were sacrificed 24 hours after dosing, 5 animals per sex received a 2000 mg/kg dose of the dosing material and were sacrificed 48 hours after dosing, and 5 animals per sex received a 2000 mg/kg dose of the dosing material and were sacrificed 72 hours after dosing (a negative control (olive oil) group of 5 animals per sex was included at each time point) and then, in a dose response micronucleus study, the dosing material was administered to 5 animals per sex per dose at dose levels of 0 (olive oil), 500, 1000, and 2000 mg/kg; these animals were sacrificed 24 hours after dosing. Bone marrow cells from the femurs were examined: polychromatic erythrocytes were evaluated for the presence of micronuclei and the ratio of polychromatic erythrocytes to normochromatic erythrocytes plus polychromatic erythrocytes was determined for each dose level. No treatment-related effects were observed. The positive control (Mitomycin C) was functional. In conclusion, the results of this study indicate that under test conditions, the test article does not induce micronuclei formation in the bone marrow cells of ICR (Crj:CD-1) mice. **No adverse effects indicated. Acceptable. (Corlett and Leung, 12/31/2010)

0045, 246015; "DNA Repair Test of Fluazinam Technical in *Bacillus subtilis*" (Ohtsuka, M. and Yamamoto, T., Hita Research Laboratories, Chemical Biotesting Center, Chemicals Inspection and Medical Testing Institute, Hita, Japan, Report No. T-1595, 10/07/1988). 870.5500. Single cultures of *Bacillus subtilis* strains H17 and M45 were exposed (disk diffusion assay) to Fluazinam technical (Lot No. 109, purity = 95.3%) at 0 (DMSO), 0.003, 0.01, 0.03, 0.1, and 0.3 ug/disk (without metabolic activation) and at 0 (DMSO), 0.3, 1, 3, 10, and 30 ug/disk (with metabolic activation) and incubated for 24 hours at 37°C. Negative and positive controls were functional. The test article exhibited the same growth inhibition against both strains of *Bacillus subtilis*. **No adverse effects indicated. Unacceptable but possibly upgradable** with the submission of a scientific justification why 1 disk/plate and not 2 disks/plates per dose level was used in this study. (Corlett and Leung, 01/05/2011)

0045, 246016; "Autoradiographic DNA Repair Test on Rat Hepatocytes" (Puri, E., Ciba-Geigy Limited, Experimental Pathology, Basel Switzerland, Test No. 840658, 11/20/1984). 870.5550. Freshly isolated cultures of male rat (Tif:RAIf(SPF)) hepatocytes were treated with CGA 143 268 techn. (Batch No. KGL 3147/5, ES 4390, purity ≥ 95%) and ³H thymidine for 5 hours at concentrations of 0 (culture medium), 0 (DMSO), 0.05, 0.25, 1.25, or 6.25 ug/ml using 2 cell cultures per dose level. Dimethylnitrosamine (DMN) was used as the positive control and was

functional. Comparison of the number of silver grains per nucleus in the negative controls and in the treated cell cultures revealed no treatment-related effect. **No adverse effects indicated. Unacceptable but possibly upgradable** with a submitted justification why only 2 independent cultures per dose level were used and with the submission of a signed GLP statement. (Corlett and Leung, 01/11/2011)

NEUROTOXICITY

Rat Acute Neurotoxicity Study

0046; 246017; "An Acute Neurotoxicity Screening Study in Rats with Technical Fluazinan (IFK-1216)" (Serrone, D.M. and Laveglia, J., Ricerca, Inc., Department of Toxicology and Animal Metabolism, Painesville, OH, Study Number 93-0075, 10/03/1995). 870.62. Technical Fluazinan (Lot Number 1030/91, purity = 96.8%), suspended in 1.5% (w/v) aqueous methylcellulose, was administered in a single dose by gavage to 10 Crl:CD BR VAF/Plus (Sprague-Dawley) rats per sex per dose at dose levels of 0 (vehicle only), 50, 1000, and 2000 mg/kg. No mortalities occurred. Cageside observations revealed no treatment-related clinical signs. No effects on body weight were observed. During FOB assessments conducted 5 to 7 hours after treatment, treatment-related soft stools were observed in both sexes at 1000 and 2000 mg/kg; this effect was not observed during FOB assessments on day 7 and on day 14. During motor activity assessments 5 to 7 hours after treatment, a treatment-related decrease in the mean counts of beam interruptions in the vertical position was observed in females at 1000 and 2000 mg/kg; this effect was not observed during motor activity assessments on day 7 and on day 14.

Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F) = 50 mg/kg (based on soft stools observed during FOB 5-7 hours after treatment). **Unacceptable but possibly upgradable** with the submission of positive control data supporting the sensitivity of FOB evaluations. (Corlett and Leung, 04/05/2010)

Rat Subchronic Neurotoxicity Studies

0047, 246018; "IKF-1216: Neurotoxicity to Rats by Dietary Administration for 13 Weeks" (Hughes, E.W., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Study Document Number ISK 251/971800, 02/10/1998). IKF-1216 (Lot no. 6109, purity = 96.9%) was admixed to the diet and fed to 10 Crl: CD BR rats per sex per dose at dose levels of 0 (untreated diet), 300, or 1000 ppm (0, 20.7, and 69 mg/kg/day, respectively, for males and 0, 23.4, and 81 mg/kg/day, respectively, for females) for 13 weeks. No mortalities occurred during the study. No treatment-related clinical signs were observed. A treatment-related decrease in mean body weight gain was observed in females at 1000 ppm. Other than the mean body weight decrease mentioned above, no treatment-related effects were observed during FOB assessments. No treatment-related effects were observed during locomotor activity assessments. Microscopic examination of nervous tissues revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M) = 69 mg/kg/day (1000 ppm) based on no effects at the highest dose tested; NOEL (F) = 23.4 mg/kg/day (300 ppm) based on decreased body weight gain. **Supplemental study** (only two dose levels were used). (Corlett and Leung, 04/15/2010)

0048, 246019; "IKF-1216: Neurotoxicity to Rats by Dietary Administration for 13 Weeks" (Hughes, E.W., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Study Document Numbers ISK 250/972895, 02/10/1998). 870.6200. IKF-1216 (Lot no. 9601-2, purity = 98.4%) was admixed to the diet and fed to 10 Crl: CD BR rats per sex per dose at dose levels of 0 (untreated diet), 1000, 2000, or 3000 ppm (0, 74, 149, and 233 mg/kg/day, respectively, for males and 0, 89, 175, and 280 mg/kg/day, respectively, for females) for 13 weeks. No mortalities occurred during the study. No treatment-related clinical signs were observed. A treatment-related decrease in mean body weight gain was observed in males at 2000 and 3000 ppm and in females at all dose levels. A treatment-related decrease in mean food consumption was observed in both sexes at 2000 and 3000 ppm. Other than the mean body weight decreases mentioned

above, walking on toes gait in males at 3000 ppm during week 4, and decreased rectal temperatures in females at 3000 during week 8 and week 13, no treatment-related effects were observed during FOB assessments. No treatment-related effects were observed during locomotor activity assessments. Microscopic examination of nervous tissues revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M) = 74 mg/kg/day (1000 ppm) based on decreased body weight gain and food consumption; NOEL (F) not determined (decreased body weight gain at all dose levels). **Acceptable.** (Corlett and Leung, 04/12/2010)

Rat Developmental Neurotoxicity Study

0049, 0050, 246020, 246021; "Technical Fluazinam: Developmental Neurotoxicity in the Rat by Oral (Gavage) Administration" (Fulcher, S.M., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Project Identity ISK/272, 03/31/2005). 870.6300. Technical Fluazinam (Lot no. A629/1995, purity = 97.8%) was suspended in 0.5% aqueous sodium carboxymethylcellulose and administered as a daily dose by gavage to 24 mated female Crl:CD (SD) IGS BR rats (F0 generation) per dose at dose levels 0 (vehicle only), 2, 10, or 50 mg/kg/day from day 6 after mating to day 20 or 21 of lactation (inclusive) and to the F1 offspring (culled to 4 males and 4 males per litter on day 4 where possible) from day 7 of age to day 21 of age (inclusive). No treatment-related clinical signs were observed in the F0 females. Treatment-related distended and dark abdomen was observed in 8 F1 offspring in one litter at 50 mg/kg/day and a distended and/or dark abdomen was observed in 4 F1 offspring (3 litters affected) at 50 mg/kg/day. Treatment-related decreases in mean body weight gain and mean food consumption were observed in F0 females during gestation at 10 and 50 mg/kg/day. A treatment-related decrease in mean body weight was observed in F1 males and females at 10 and 50 mg/kg/day throughout during the 63-day observation period. FOB and motor activity assessments on F0 females revealed no treatment-related abnormalities. FOB and motor activity assessments on F1 males and F1 females revealed no consistent effects on these animals. Auditory startle response habituation assessments on F0 females revealed no treatment-related abnormalities. A dose-related decrease in mean auditory startle response habituation peak amplitude during trials 11-50 on day 23/24 in F1 males at 50 mg/kg/day, a dose-related increase in mean auditory startle response habituation latency to peak during trials 41-50 on day 23/24 in F1 females at 50 mg/kg/day, and a dose-related decrease in mean auditory startle response pre-pulse inhibition peak amplitude (stimulus with and without pre-pulse) on day 23/24 in F1 males at 50 mg/kg/day were observed. Assessments of learning and memory using a Morris maze revealed no treatment-related effects in F0 females or in F1 males and females. Macroscopic and microscopic examinations of the nervous system and brain morphometry measurements revealed no treatment-related abnormalities in the F0 females or in the F1 males and females. **No adverse effects. Reported Maternal NOEL** = 2 mg/kg/day (based on decreased mean maternal weight gain and mean food consumption), **Reported Developmental NOEL** = 2 mg/kg/day (based on decreased mean pup body weight). **Unacceptable but possibly upgradable** with submission of positive control data demonstrating the sensitivity of the procedures used. (Corlett and Leung, 06/10/2010)

0050, 254039; "Technical Fluazinam: Preliminary Developmental Neurotoxicity Study by Oral Gavage Administration to CD Rats and Their Offspring" (Fulcher, S.M., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Project Identity ISK/271/040029, 11/03/2005). Technical Fluazinam (Lot no. A629/1995, purity = 97.8%) was suspended in 0.5% aqueous sodium carboxymethylcellulose and administered as a daily dose by gavage to 7 mated female Crl:CD (SD) IGS BR rats (F0 generation) per dose at dose levels 0 (vehicle only), 10, 50, 100, or 200 mg/kg/day from day 6 after mating to day 20 of lactation (inclusive with 2 animals per dose level sacrificed on day 8 of lactation leaving 5 per dose level) and to the F1 offspring (culled to 4 males and 4 males per litter on day 4 where possible from the 5 females per dose level) from day 7 of age to day 20 of age (inclusive). No maternal mortalities occurred during the study; offspring at 200 mg/kg/day were sacrificed between days 9 and 11 of age and offspring at 100 mg/kg/day were sacrificed on days 20 or 21 of age due to adverse effects while offspring at 0, 10, and 50

mg/kg/day were sacrificed at day 21 of age. No treatment-related clinical signs were observed in the F0 females. Treatment-related swollen/distended and dark abdomen and red discharge from the anus were observed in the F1 offspring from multiple litters at 100 and 200 mg/kg/day and in the F1 offspring from one litter at 50 mg/kg/day. A treatment-related decrease in mean body weight gain in F0 females during gestation days 6-20 at 200 mg/kg/day and during lactation days 1-21 at 100 mg/kg/day was observed. A treatment-related decrease in mean food consumption in F0 females during lactation days 17-20 at 100 mg/kg/day was observed. A treatment-related decrease in mean body weight gain was observed in F1 males and females from days 7-21 of age at 50 and 100 mg/kg/day. **No adverse effects indicated. Reported Maternal NOEL = 50 mg/kg/day** (based on decreased mean maternal body weight gain), **Reported Developmental NOEL = 10 mg/kg/day** (based on decreased mean pup body weight gain). **Supplemental study** (only 7 animals per dose level were used, and no FOB, motor activity assessments, and histopathology on nervous system tissues were performed). (Corlett and Leung, 07/14/2010)

Comparative Neurotoxicity Evaluation in Mice, Rats and Dogs

0051, 246022; "B-1457: Comparative Study on Susceptibility to Neurotoxicity in Mice, Rats, and Dogs" (Nakashima, N., The Institute of Environmental Toxicology, Tokyo, Japan, Laboratory Project ID: IET 98-0020, 06/22/1998). B-1457 (Lot no. 9604, purity = 97%) was suspended in 0.5% carboxymethyl cellulose sodium salt solution and administered orally by gavage once per day for 3 consecutive days to 5 male ICR (Crj:CD-1) mice and to 5 male Sprague-Dawley [Crj:CD(SD)] rats per dose at dose levels of 0 (vehicle only) and 2.0 mg/kg. Also, the suspended test article was administered orally by gavage to 3 male beagle dogs for 3 consecutive days at dose levels of 0 (vehicle only) and 2.0 mg/kg with each daily dose divided into halves that were administered one hour apart in an attempt to prevent the dogs from vomiting. No animals died. All animals were sacrificed 1 day after the last administered dose, necropsies were performed, brain weight was measured and histopathological examinations were performed on brain and liver tissue. No treatment-related clinical signs were observed in the treated dogs; a decrease in spontaneous motor activity was observed in 3 of the 5 treated mice; decreased spontaneous motor activity, bradypnea, and lateral recumbent posture were observed in treated rats. A treatment-related decrease in mean body weight was observed in the mouse and the rat; no effect was observed in the dog. A treatment-related increase in mean absolute brain weight was observed in the rat and the mouse but not the dog. Macroscopic examination revealed treatment-related swelling of the brain in rats but not in mice or dogs. Histopathological examination revealed treatment-related slight white matter vacuolation in the brain of the mice and rats and trace white matter vacuolation in the brain of the dogs; no liver effects were observed in any species. **Possible adverse effect indicated:** swelling in the brain in rats and slight white matter vacuolation in the brain in rats and mice. NOEL not determined. **Supplemental study** (non-guideline study). (Corlett and Leung, 04/20/2010)

Rat Neurotoxicity Evaluation

0051, 246023; "Fluazinam Technical: Toxicological Effect on Brain of Rats and Its Reversibility by Dietary Administration for 14 Days Followed by a 25-Day Recovery Period" (Nomura, M., Environmental Sciences Group, Regulatory Affairs Division, Biosciences General Headquarters, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan, ES-ISK Study Number: AN-1323, 06/12/1998). Fluazinam technical (Lot no. 1030/91, purity = 96.2%) was admixed to the diet and fed to 7 male Crj: CD (SD) SPF/VAF rats per dose at dose levels of 0 (untreated diet), 10,000, or 30,000 ppm (0, 714, and 1743 mg/kg/day, respectively) for 14 consecutive days. 4 animals at each dose level were sacrificed after 14 days of treatment, the remaining 3 animals at each dose level were fed the control diet for 25 days (recovery period), and the recovery animals were sacrificed at the end of this recovery period. In rats receiving 30,000 ppm, coarse fur was observed in all during the treatment period beginning on day 4 of treatment and emaciation was observed in all on days 5 and 6 of treatment with the coarse fur sign clearing in the recovery group animals by day 4 of the recovery period. In rats receiving 10,000 ppm, coarse fur was observed in all during the treatment period beginning on day 5 of treatment with the coarse fur sign clearing in the recovery

group animals by day 4 of the recovery period. A statistically significant decrease in mean body weight was observed at 10,000 and 30,000 ppm during the treatment period; during the recovery period body weight differences between the treated animals and the controls ceased to be statistically significant. A statistically significant decrease in mean food consumption was observed at 10,000 and 30,000 ppm during the treatment period; within a week during the recovery period, the food consumption values of the treated animals were similar to those of the control animals. No treatment-related effects on brain weight were observed. Macroscopic examination revealed enlarged liver, pale liver, and accentuated lobular pattern in the liver in the treated animals at 10,000 and 30,000 ppm sacrificed after 14 days; none of these effects were observed in the sacrificed recovery group animals. Macroscopic examination also revealed trace edema in the brains of all the treatment period animals at 30,000 ppm; this was not observed in the recovery group animals. Histopathological examination revealed treatment-related slight to moderate white matter vacuolation in the brain at 30,000 ppm and trace white matter vacuolation in the brain at 10,000 ppm in animals sacrificed after the treatment period, and trace white matter vacuolation in the brain at 30,000 ppm in the recovery group animals. **Possible adverse effect indicated:** white matter vacuolation in the brain. NOEL not determined. **Supplemental study** (non-guideline study). (Corlett and Leung, 04/22/2010)

Mouse Neurotoxicity Evaluation

0051, 246024; "Fluazinam Technical: Toxicological Effect on Brain of Mice and Its Reversibility by Dietary Administration for 4 or 28 Days Followed by a 56-Day Recovery Period" (Nomura, M., Environmental Sciences Group, Regulatory Affairs Division, Biosciences General Headquarters, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan, ES-ISK Study Number: AN-1333, 06/12/1998). Fluazinam technical (Lot no. 1030/91, purity = 96.2%) was admixed to the diet and fed to groups of 5 male Crj: CD-1 (ICR) SPF/VAF mice per dose per treatment period per recovery period. For the treatment period of 4 days, dose levels of 0, 7,000, or 20,000 ppm (0, 1173, or 1871 mg/kg/day, respectively) and recovery periods of 0, 7, 14, 24, and 56 days were used. For the treatment period of 28 days, dose levels of 0 or 7,000 ppm (0 and 1043 mg/kg/day, respectively) and recovery periods 0, 28, and 56 days were used. No mortalities occurred. At the 20,000 ppm dose level, the following assessments were made during FOB after 4 days of treatment: hunched posture in 1 animal, rough fur and unkempt appearance in 1 animal, slightly impaired mobility in 2 animals, Straub tail in 1 animal, no unsupported rearing in 5 animals, and land foot splay wider than control animals in 2 animals; no abnormalities were observed during FOB assessments at the next observation (day 7 of the recovery period). A treatment-related decrease in mean body weight was observed at 20,000 ppm in animals treated for 4 days and at 7,000 ppm in animals treated for 28 days with mean body weights ceasing to be statistically significant when compared to the controls on recovery day 14 and on recovery day 7, respectively. No treatment-related effects on brain weight were observed. Macroscopic examination revealed enlarged liver, pale liver, and accentuated lobular pattern in the liver in the animals treated for 4 days at 7,000 and 20,000 ppm and in the animals treated for 28 days at 7000 ppm sacrificed after 14 days with none of these effects observed in the recovery group animals sacrificed on recovery day 7 and recovery day 28, respectively. Macroscopic examination also revealed trace edema in the brains of all 5 animals treated for 4 days at 20,000 ppm and of 2 of 5 animals treated for 28 days at 7,000 ppm with this effect not observed in the recovery group animals sacrificed on recovery day 7 and recovery day 28, respectively. Histopathological examination of animals treated for 4 days revealed treatment-related white matter vacuolation in the brain at 7,000 ppm (trace to minimal) and at 20,000 ppm (minimal to moderate) with this effect persisting in the recovery group animals (recovery days 7 and 14 for the animals at 7,000 ppm and recovery days 7, 14, and 24 for animals at 20,000 ppm) and finally clearing in all treated animals by recovery day 56. Histopathological examination of animals treated for 28 days revealed treatment-related white matter vacuolation in the brain at 7,000 ppm (minimal to marked) with this effect persisting in the recovery group animals on recovery day 28 but clearing in recovery group animals on recovery day 56. **Possible adverse effect indicated:** white matter vacuolation in the brain. NOEL not determined. **Supplemental study** (non-guideline study). (Corlett and Leung, 04/28/2010)

0051, 246025; "Fluazinam Technical and Analytical Standard of Fluazinam: Toxicological Effect on Male Mice Following a Single or Repeated Oral Administration" (Nomura, M., Environmental Sciences Group, Regulatory Affairs Division, Biosciences General Headquarters, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan, ES-ISK Study Numbers: AN-1318 and AN-1324, 06/12/1998). Two studies were conducted. Fluazinam technical (Lot no. 1030/91, purity = 97.0% and 8412-20, purity = 95.3%) and Analytical Standard of Fluazinam (Lot no. Y910401, purity = 99.7%) were used as test articles. In study no. AN-1318, Fluazinam technical (Lot no. 1030/91, purity = 97.0%) was prepared in corn oil and administered by gavage to 5 male Crj:CD-1 (ICR) SPF/VAF mice at a dose level of 3000 mg/kg on 2 consecutive days and administered once by gavage to 5 male Crj:CD-1 (ICR) SPF/VAF mice at a dose level of 5000 mg/kg; Fluazinam technical (Lot no. 8412-20, purity = 95.3%) was administered in the same manner to the same number animals using the same dose levels as Fluazinam technical (Lot no. 1030/91) above; 5 animals were administered corn oil only as above. In study no. AN-1324, Analytical Standard of Fluazinam (Lot no. Y910401, purity = 99.7%) was prepared in corn oil and administered by gavage in a single dose to 5 male Crj:CD-1 (ICR) SPF/VAF mice at a dose level of 5000 mg/kg (2 of the animals were from the same colony as those used in Study no. AN-1318 and the other 3 animals used were older animals). In animals dosed with Fluazinam technical Lot no. 1030/91, mortalities occurred as follows within 48 hours: 3/5 and 4/5 for 3000 mg/kg and 5000 mg/kg, respectively. In animals dosed with Fluazinam technical Lot no. 8412-20, mortalities occurred as follows within 48 hours: 1/5 and 1/5 for 3000 mg/kg and 5000 mg/kg, respectively. No mortalities occurred in animals dosed with Analytical Standard of Fluazinam. All surviving animals were sacrificed 48 hours after the commencement of treatment. Treatment-related clinical signs observed in animals dosed with Fluazinam technical (both lot numbers and both dose levels) included prone position, paralysis of hind legs, decreased locomotor activity, tremor, and moribund state. No clinical signs were observed in animals treated with Analytical Standard of Fluazinam except for course fur in one animal at 20 and 24 hours after dosing with this sign clearing 48 hours after dosing. A treatment-related decrease in mean body weight was observed in animals treated with Fluazinam technical (both lot numbers and dose levels); no effect on body weight was observed in animals treated with Analytical Standard of Fluazinam. A statistically significant increase in mean brain weight was observed in animals treated with Fluazinam technical (both lot numbers and dose levels); this effect was not observed in animals treated with Analytical Standard of Fluazinam. Macroscopic examination revealed treatment-related trace to marked edema in the brains of all animals treated with Fluazinam technical (both lot numbers and dose levels). Histopathological examination of all animals treated with Fluazinam technical revealed treatment-related minimal to marked white matter vacuolation in the brain (both lot numbers and dose levels). Macroscopic and histopathological examinations of all animals treated with Analytical Standard of Fluazinam revealed no signs of brain pathology. **Possible adverse effect indicated:** white matter vacuolation in the brain in animals treated with Fluazinam technical. NOEL not determined for animals treated with Fluazinam technical. NOEL (M) = 5000 mg/kg for animals treated with Analytical Standard of Fluazinam (based on no treatment-related effects at the highest dose tested). **Supplemental study** (non-guideline study). (Corlett and Leung, 05/03/2010)

Neurotoxicity of Impurities

0051, 246026; "Various Impurities in Fluazinam Technical: Toxicological Effect on Brain of Mice Following a Single Oral Administration" (Nomura, M., Environmental Sciences Group, Regulatory Affairs Division, Biosciences General Headquarters, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan, ES-ISK Study Numbers: AN-1375, AN-1411, and AN-1486, 06/12/1998). Three studies were conducted. Test articles used were, in Study No. AN-1375 Impurity-2 (no lot number, purity = 98.9%), impurity-3 (Lot No. 9105, purity = 99.2%), Impurity-8 (Lot No. 9507, purity = 100%), and Impurity-7 (Lot No. Y950801, purity = 100%), in Study No. AN-1411, Impurity-1 (Lot No. 8406-3, purity = 96.0%), Impurity-4 (Lot No. 900726, purity = 99.5%), Impurity-6 (Lot No. 8806, purity = 99.6%), and Impurity-9 (Lot No. 9105, purity = 99.2%), and in Study No. AN-1486, Impurity-5 (Lot No. Y950807, purity = 99.5%). In each study, the test articles were prepared in corn oil and

administered by gavage in a single dose to 5 male Crj:CD-1 (ICR) SPF/VAF mice at a dose level of 50 mg/kg except for Impurity-1 where 2 groups of 5 mice and dose levels of 20 and 200 mg/kg were used, Impurity-9 where a dose level of 100 mg/kg was used, and Impurity-5 where a dose level of 5 mg/kg was used. A group of 3 to 5 mice treated with corn oil only was included in each study. No mortalities occurred except for the animals treated with Impurity-5 where all the animals were sacrificed *in extremis* 24 hours after treatment. All surviving animals were sacrificed 48 hours after treatment. No clinical signs were observed except in the animals treated with Impurity-5 where 3 of the 5 animals exhibited coarse fur, paralysis of the hind legs, and staggering gait and the other 2 animals exhibited sedation and were considered moribund. No effect on body weight was observed except for a treatment-related decrease in mean body weight in animals treated with Impurity-5. A statistically significant increase in mean brain weight was observed in animals treated with Impurity-5; this effect was not observed in animals treated with the other test articles. Macroscopic examination revealed treatment-related trace to moderate edema in the brains of 4 of 5 animals treated with Impurity-5; this effect was not observed in the animals treated with the other test articles. Histopathological examination of the animals treated with Impurity-5 revealed treatment-related trace to moderate white matter vacuolation in the brains of all test animals; this effect was not observed in the animals treated with the other test articles. **Possible adverse effect indicated:** white matter vacuolation in the brain of animals treated with Impurity-5. NOEL not determined for animals treated with Impurity-5. NOEL (M) for Impurity-2, Impurity-3, Impurity-8, Impurity-7, Impurity-4, Impurity-6 = 50 mg/kg (based on no treatment-related effects at the highest dose tested). NOEL (M) for Impurity-1 = 200 mg/kg (based on no treatment-related effects at the highest dose tested). NOEL (M) for Impurity-9 = 100 mg/kg (based on no treatment-related effects at the highest dose tested). **Supplemental study** (non-guideline study). (Corlett and Leung, 05/04/2010)

0051, 246027; "Impurity-5, an Impurity in Fluazinam Technical: Sensitivity Comparison on Brain of Mice and Rats Following 14 Day Oral Administrations" (Nomura, M., Environmental Sciences Group, Regulatory Affairs Division, Biosciences General Headquarters, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan, ES-ISK Study Number: AN-1481, 06/12/1998). Impurity-5 (Lot No. Y950807, purity = 99.5%) was prepared in 0.5% sodium carboxymethyl cellulose and administered orally daily for 14 days to 7 female Crj:CD-1 (ICR) SPF/VAF mice at dose levels of 0 (vehicle only) and 0.5 mg/kg and to 7 female Crj:CD (SD) SPF/VAF rats at dose levels of 0 (vehicle only) and 0.5 mg/kg. No mortalities occurred in either species. No clinical signs were observed in either species. No effects on body weight were observed in either species. No effect on brain weight was observed in either species. Macroscopic examination revealed no abnormalities in either species. Histopathological examination revealed treatment-related trace white matter vacuolation in the brains of all animals of both species. **Possible adverse effect indicated:** white matter vacuolation in the brain of both mice and rats. NOEL not determined. **Supplemental study** (non-guideline study; only females were used and only one dose was used). (Corlett and Leung, 05/05/2010)

0051, 246028; "Impurity-5, an Impurity in Fluazinam Technical: Sensitivity Comparison on Brain of Rats and Mice in Three and 10 Weeks Old Following 14 Day Oral Administrations" (Nomura, M., Environmental Sciences Group, Regulatory Affairs Division, Biosciences General Headquarters, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan, ES-ISK Study Number: AN-1492, 06/12/1998). Impurity-5 (Lot No. Y950807, purity = 99.5%) was prepared in 0.5% sodium carboxymethyl cellulose and administered orally daily for 14 days to 5 male 3-week old and 5 male 10-week old Crj:CD-1 (ICR) SPF/VAF mice each at dose levels of 0 (vehicle only) and 0.5 mg/kg) and to 5 male 3-week old and 5 male 10-week old Crj:CD (SD) SPF/VAF rats each at dose levels of 0 (vehicle only) and 0.5 mg/kg. No mortalities occurred in either species at either age group. No clinical signs were observed in either species at either age group. No effects on body weight were observed in either species at either age group. No effect on brain weight was observed in either species at either age group. Macroscopic examination of the brain revealed treatment-related trace edema in the brain of 1 treated 10-week old rat and in the brain of 2

treated 10-week old mice; no other abnormalities in either species were observed. Histopathological examination of the brain revealed treatment-related trace to moderate white matter vacuolation in the brains of 1 3-week old rat and 2 3-week old mice and in all treated 10-week old rats and mice. **Possible adverse effect indicated:** white matter vacuolation in the brain of both mice and rats. NOEL not determined. **Supplemental study** (non-guideline study; only males were used and only one dose was used). (Corlett and Leung, 05/06/2010)

0051, 246029; "Impurity-5, an Impurity in Fluazinam Technical: Toxicological Effect on Brain and Optic Nerves of Mice Following a Single Oral Administration at Various Stages of Animal Age" (Nomura, M., Environmental Sciences Group, Regulatory Affairs Division, Biosciences General Headquarters, Ishihara Sangyo Kaisha, Ltd., Shiga-ken, Japan, ES-ISK Study Number: AN-1480, 06/12/1998). Impurity-5 (Lot No. Y950807, purity = 99.5%) was prepared in an aqueous solution of 0.5% carboxymethyl cellulose sodium salt and administered by gavage in a single dose to 8 different age groups of mice each consisting of 5 male Crj:CD-1 (ICR) mice dosed with 2.5 mg/kg. The age groups treated were 3, 5, 8, 10, 12, 16, 20, and 24 weeks of age. All animals were sacrificed 48 hours after treatment. No mortalities occurred in any age group. No clinical signs were observed in any age group. No apparent effects on body weight were observed in any age group (no control groups included in the study). No apparent effect on brain weight was observed in any age group (no control groups included in the study). Macroscopic examination revealed no abnormal findings except for trace edema in the brain of one animal in the 10 weeks of age group. Histopathological examination of the brain revealed treatment-related trace white matter vacuolation in 1, 2 and 3 treated animals in the 3-week, 5-week, and 8-week age groups, respectively, and trace to slight treatment-related white matter vacuolation in all of the animals in the other age groups. Histopathological examination of the optic nerves revealed no vacuolation in the optic nerves in the 3-week age group, trace vacuolation in the optic nerves in 1 animal in both the 5-week and 8-week age groups, and trace vacuolation in the optic nerves in 2 to 4 animals in the other age groups. **Possible adverse effect indicated:** white matter vacuolation in the brain and vacuolation of the optic nerves. NOEL not determined. **Supplemental study** (non-guideline study; only males were used, only one dose level was used, and no negative control groups were used). (Corlett and Leung, 05/10/2010)

SUBCHRONIC STUDIES

Rat 4-Week Dietary Toxicity Study

0028, 245970; "B-1216: Four-Week Toxicity Study in Dietary Administration to CD Rats Final" (Broadmeadow, A., Life Science Research, Stock, Essex, England, LSR Report No. 83/ISK035/544, 05/23/1983). 870.3050. B-1216 (Lot number 8203, purity = 96.3%) was admixed to the diet and fed to 10 CD (remote Sprague-Dawley) rats per sex per dose at dose levels of 0 (untreated diet), 10, 50, 250 or 3000 ppm (0, 1.0, 5.1, 26.3, and 302 mg/kg/day, respectively for males and 0, 1.0, 5.3, 25.8, and 308 mg/kg/day, respectively for females, calculated by the reviewer) for 4 weeks. No animals died during the study. No treatment-related clinical signs were observed. Treatment-related decreases in mean body weight gain and food consumption were observed in both sexes at 3000 ppm. A treatment-related decrease in mean hemoglobin concentration was observed in both sexes at 3000 ppm. A treatment-related increase in mean the total cholesterol level was observed in both sexes at 250 and 3000 ppm and a treatment-related increase in the mean phospholipid level was observed in males at 3000 ppm and in females at 250 and 3000 ppm. A treatment-related increase in mean relative liver weight in males at 50, 250, and 3000 ppm and in females at 250 and 3000 ppm was observed. Microscopic examination revealed a treatment-related increase in periportal hypertrophy in the liver in males at 250 and 3000 ppm and occasional single cell necrosis with mononuclear infiltration in the liver in females at 3000 ppm. **No adverse effects.** NOEL (M) = 1.0 mg/kg/day (10 ppm) and NOEL (F) = 5.1 mg/kg/day (50 ppm) based on increased relative liver weights and liver histopathology. **Acceptable.** (Corlett, 02/09/2010)

Rat Subchronic Dietary Toxicity Studies

0029, 245971, 245972; "B-1216: 13-Week Toxicity Study in Dietary Administration to CD Rats, Amended Final Report" (Broadmeadow, A., Life Science Research Limited, Eye, Suffolk, England, original LSR Report No. 84/ISK046/635, amended final report no. 91/ISK046/0830, 11/07/1991). 870.3100. B-1216 (Lot no. 8303-2, purity = 98.5%) was admixed to the diet and fed to 10 CD (remote Sprague-Dawley origin) rats per sex per dose at dose levels of 0 (untreated diet), 2, 10, 50, or 500 ppm (0, 0.15, 0.77, 3.8, and 38 mg/kg/day, respectively for males and 0, 0.17, 0.86, 4.3, and 44 mg/kg/day, respectively for females, calculated by the reviewer) for 13 weeks. No treatment-related mortalities occurred during the study. No treatment-related clinical signs were observed. No treatment-related effects on body weight and food consumption were observed. Treatment-related decreases in mean hemoglobin, mean hemoglobin, and hematocrit concentrations were observed in males at 500 ppm after 12 weeks of treatment. Serum chemistry and urinalysis revealed no treatment-related effects. No effect on hepatic microsomal aminopyrine-N-demethylase activity was observed. Treatment-related increases in mean relative liver weight in males at 500 ppm and in mean relative lung weight in females at 500 ppm were observed. Microscopic examination revealed treatment-related increases in sinusoidal chronic inflammation and periportal hypertrophy in the liver in males at 500 ppm and a treatment-related hepatitis in females at 500 ppm. **No adverse effects.** NOEL (M) = 3.8 mg/kg/day (50 ppm) based on sinusoidal chronic inflammation and periportal hypertrophy in the liver and NOEL (F) = 4.3 mg/kg/day (50 ppm) based on hepatitis. **Acceptable.** (Corlett, 02/17/2010)

0030, 245973; "B-1216: 13-Week Toxicity and 4-Week Reversibility Study in Dietary Administration to CD Rats" (Broadmeadow, A., Life Science Research Limited, Eye, Suffolk, England, original LSR Report No. 84/ISK045/581, amended final report no. 91/ISK045/1037, 11/07/1991). B-1216 (Lot no. 8303-2, purity = 98.5%) was admixed to the diet and fed to 10 CD (remote Sprague-Dawley origin) rats per sex per dose at dose levels of 0 (untreated diet) and 500 ppm (0 and 37.63 g/kg/day, respectively for males and 0 and 44.71 mg/kg/day, respectively for females, calculated by the reviewer) for 13 weeks [10 additional rats per sex per dose level were included to assess reversibility (4-week recovery period used)]. No animals died during the study interval. No treatment-related clinical signs were observed. No treatment-related effects on body weight and food consumption were observed. No effect on hepatic microsomal aminopyrine-N-demethylase activity was observed. A treatment-related increase in mean relative liver weight was observed in animals sacrificed after 13 weeks of treatment (both sexes) at 500 ppm; this effect was not observed in the 4-week recovery group animals. Microscopic examination revealed a treatment-related increase in periportal hepatocytic hypertrophy in males sacrificed after 13 weeks of treatment at 500 ppm; this effect was not observed in the 4-week recovery group animals. **No adverse effects.** NOEL not determined (increased mean relative liver weights at 500 ppm). **Supplemental study** (only 1 dose level was used and no hematology and no serum chemistry were performed). (Corlett, 02/23/2010)

Rat 21-Day Repeated Dosing Dermal Toxicity Study

0033; 245977; "B-1216: 21-Day Percutaneous Toxicity Study in CD Rats, Amended Final Report" (Cummins, H.A., Life Science Research Limited, Eye, Suffolk, England, original LSR Report No. 84/ISK052/690, amended final report no. 91/ISK052/0824, 11/07/1991). 870.3200. B-1216 (Lot 8303-2, purity = 98.5%), mixed with 0.5% methylcellulose in distilled water, was applied to the clipped dorsal region of 10 CD (remote Sprague-Dawley origin) rats per sex per dose at dose levels of 0 (vehicle only), 10, 100, or 1000 mg/kg/day for 6 hours daily for 21 days. No treatment-related mortalities occurred. No clinical signs were observed. Erythema was observed at the treated sites at all dose levels. A treatment-related decrease in weight gain was observed in males at 1000 mg/kg/day. Treatment-related increases in mean aspartate aminotransferase and total cholesterol levels in males at 100 and 1000 mg/kg/day and in females at 1000 mg/kg/day. A treatment-related increase in mean relative liver weight was observed in both sexes at 1000 mg/kg/day. Microscopic examination revealed periportal hepatocytic hypertrophy in both sexes at 1000 mg/kg/day and in one male at 100 mg/kg/day. **No adverse effects indicated.**

Reported NOEL (M, systemic) = 10 mg/kg/day and NOEL (F, systemic) = 100 mg/kg/day based on increases in mean aspartate aminotransferase and total cholesterol levels, an increase in mean relative liver weight and periacinar hepatocytic hypertrophy, and NOEL (M/F, skin) < 10 mg/kg/day (erythema in rats treated at the lowest dose). **Acceptable.** (Corlett and Leung, 03/22/2010)

Dog Subchronic Oral Toxicity Studies

0031, 245974, 245975; "B-1216: 13-Week Toxicity Study in Oral Administration to Beagle Dogs, Amended Final Report" (Broadmeadow, A., Life Science Research Limited, Eye, Suffolk, England, original LSR Report No. 84/ISK048/692, amended final report no. 91/ISKF048/0832, 11/07/1991). 870.3150. B-1216 (Lot no. 8303-2, purity = 98.5%) was administered by gelatin capsule to 4 beagle dogs per sex per dose at dose levels of 0 (one empty gelatin capsule), 1, 10, and 100 mg/kg/day once a day 7 days a week for at least 13 weeks. No animals died during the treatment interval. No clinical signs were reported. No clear treatment-related effects on body weight and food consumption were observed. Hematological examination revealed no treatment-related effects. A treatment-related increase in the mean alkaline phosphatase level was observed in females at 100 mg/kg/day. Ophthalmoscopy revealed treatment-related grey mottling in the tapetal fundus and slight hyperreflection in the tapetal fundus of the eyes in all males and females at 100 mg/kg/day. Treatment-related increases in mean relative liver weight in males and females at 100 mg/kg/day. Microscopic examination revealed treatment-related bile duct hyperplasia with/without cholangiofibrosis in both sexes at 100 mg/kg/day. **Possible adverse effect:** grey mottling in the tapetal fundus and slight hyperreflection in the tapetal fundus of the eyes at 100 mg/kg/day. NOEL (M/F) = 10 mg/kg/day based on bile duct hyperplasia with/without cholangiofibrosis in the liver. **Acceptable.** (Corlett, 03/02/2010)

0032, 245976; "B1216: Eleven Week Oral Toxicity Study in Dogs to Investigate Possible Changes in Retinal Function and Morphology and the Reversibility of Such Changes" (Hull, R.M., Imperial Chemical Industries PLC, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England, Study Number TKD/405, 09/01/1986). B-1216 (Lot no. 8303-2, purity = 98.3%) was administered by gelatin capsule to 6 male Alderley Park Beagle dogs per dose at dose levels of 0 (empty gelatin capsules) and 200 mg/kg/day (reduced to 150 mg/kg/day due to signs of toxicity in weeks 3-5) for 11 weeks at which time 3 animals from each group were terminated; the 3 remaining animals in each group were continued on the compound withdrawal phase of the study for additional 5 weeks. No animals died during the study interval. Treatment-related loose/liquid stools were observed during the treatment period, a condition that decreased to background levels during the withdrawal phase of the study. Treatment-related decreases in both in body weight gain and food consumption were observed during the treatment phase of the study; body weight gain and food consumption returned to background levels during the withdrawal phase of the study. Treatment-related increases in alanine aminotransferase and alkaline phosphatase levels were observed during the treatment phase of the study; these levels returned to background levels during the withdrawal phase of the study. Indirect ophthalmoscopy revealed increased brown granularity of the tapetal fundus in one treated animal in the non-withdrawal group starting on day 14 and continuing throughout the treatment period and possible increased brown granularity of the tapetal fundus in one treated animal in the withdrawal group starting on day 21, continuing throughout the treatment period, and becoming less apparent in the 4th week after compound withdrawal (day 104). Electroretinography (ERG) indicated a treatment-related decrease in the amplitude of the b-wave and this decrease in ERG voltage was almost completely reversed in 2 of 3 dogs by the end of the compound withdrawal interval. The ERG results are considered ambiguous. Histopathology and electron microscopy on the eyes revealed no treatment-related findings. **No adverse effects indicated by the data presented.** NOEL not determined. **Supplemental study** (retinal function and morphology study). (Corlett, 03/15/2010)

Mouse 4-Week Dietary Toxicity Studies

0026, 245968; "B-1216: Four Week Toxicity Study in Mice Final Report" (Amyes, S.J. et al., Life Science Research, Stock, Essex, England, LSR Report No. 83/ISK036/067, 06/09/1983). 870.3050. B-1216 (Lot number 8203, purity = 96.3%) was admixed to the diet and fed to 10 CD-1 mice per sex per dose at dose levels of 0 (untreated diet), 10, 50, 250 or 3000 ppm (0, 1.5, 7.6, 36, and 438 mg/kg/day, respectively for males and 0, 1.6, 8.2, 43, and 472 mg/kg/day, respectively for females) continuously for 4 weeks. No treatment-related clinical signs were observed. A treatment-related decrease in mean body weight gain was observed in males at 250 and 3000 ppm. A treatment-related increase in mean total cholesterol level was observed in both sexes at 3000 ppm. Treatment-related increases in mean relative liver weight in both sexes at 3000 ppm and in mean relative kidney weight in females at 250 and 3000 ppm were observed. Microscopic examination revealed a treatment-related increase in periportal hepatocytic hypertrophy at all doses in males and at 3000 ppm in females. **No adverse effects.** NOEL (M) not determined (treatment-related increased periportal hepatocytic hypertrophy at 10 ppm), NOEL (F) = 8.2 mg/kg/day (50 ppm) based on increased relative liver and kidney weights and increased periportal hepatocytic hypertrophy. **Acceptable.** (Corlett, 01/28/2010)

0027, 245969; "Technical Fluazinam Toxicity to Mice by Dietary Administration for 4 Weeks (According to JMAFF Guidelines)" (Chambers, P.R. et al., Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England, Report No. 49/921049, 03/07/1994). 870.3050. Technical fluazinam (Lot 1030/91, purity = 97.0%) was admixed to the diet and fed to 14 Crl:CD-1 (ICR) BR mice per sex per dose at dose levels of 0 (untreated diet), 3000, 5000, and 7000 ppm (0, 555, 938, and 1199 mg/kg/day, respectively for males and 0, 658, 1050, and 1404 mg/kg/day, respectively for females) for 4 weeks. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. No effects on body weight were observed. A treatment-related increase in liver weight adjusted to body weight (determined by analysis of covariance) was observed in both sexes at 3000, 5000, and 7000 ppm. Microscopic examination revealed a treatment-related centrilobular hepatocyte enlargement at all dose levels in both sexes. **No adverse effects.** NOEL (M/F) < 3000 ppm (M: 555 mg/kg/day; F: 658 mg/kg/day) (treatment-related centrilobular hepatocyte enlargement at all dose levels). **Acceptable.** (Corlett, 02/03/2010)

Rat 7-Day Inhalation Toxicity Study

0025, 245967; "Frowncide WP: Preliminary Toxicity Study by Inhalation to CD Rats for Seven Days" (Cracknell, S., Life Science Research Limited, Eye, Suffolk, England, LSR Report No. 90/ISK153/1390, 01/07/1992). Frowncide WP (Batch no. 004, 51.9% a.i.) was aerosolized and administered in a nose-only manner to 5 CD rats per sex per dose at dose levels (mean gravimetric concentration) of 0 (air only), 0.0028, 0.0110, 0.0322, and 0.1099 mg/l (mass median equivalent aerodynamic diameter (geometric standard deviation) of -, 3.98 (2.24), 3.85 (2.69), 3.22 (2.09), and 3.69 (2.04) μ m, respectively) over two 3-hour exposure periods each day for 7 days. No animals died during the study interval. No treatment-related clinical signs were observed. No treatment-related effect on body weight was observed. No treatment-related hematological effects were observed. No toxicological-related effects on serum chemistry were observed. Statistically significant increases in mean absolute lung weight in males and females at 0.1099 mg/l and in mean absolute testes weight at 0.0322 and 0.1099 mg/l were observed; statistically significant increases in mean relative lung and testes weight in males at 0.1099 mg/l were observed. Macroscopic examination revealed no treatment-related abnormalities. **No adverse effects.** Based on the data presented, a NOEL cannot be assigned. **Supplemental study** (not a guideline study). (Corlett, 02/24/2011)

METABOLISM STUDIES

Metabolism, Rat

0052; 246032; "Pilot Study to Evaluate Excretion of Radiolabel Following A Single Oral Dose of 14 C-IKF-1216 to Rats" (Liu, Y. et al., Ricerca, Inc., Department of Toxicology and Animal

Metabolism, Painesville, OH, Study number 92-0034, 08/06/1993). 870.7485. Two test articles were used in this study (^{14}C label in two positions of IKF-1216, Lot #0201, 99.9%): 1. [pyridine- ^{14}C]-IKF-1216, Ref. 91-66, purity = 98.5%, specific activity 1.754 Gbq/mmol and 2. [phenyl- ^{14}C]-IKF-1216, Ref. 91-67, purity= 99.5%, specific activity 2.109 Gbq/mmol. Suspensions of the two test articles in 0.75% (w/v) methylcellulose were administered by gavage to 3 Sprague-Dawley Crl:CDBR VAF/Plus rats per sex per dose per label position at dose levels of 0.5 and 50 mg/kg. In the case of the 50 mg/kg dose, for both labeled compounds, a homogeneous mixture of labeled and non-labeled IKF-1216 was prepared, while, in the case of the 0.5 mg/kg dose, only the labeled compounds were used. Each animal received between 55.3 and 72.6 uCi/kg of radioactivity. One animal of each sex per label group was dosed with the vehicle only. ^{14}C excretion into the expired air was negligible for both dosing articles. Fecal and urine samples were collected over 48 hours after the administration of the dosing compounds. For [phenyl- ^{14}C]-IKF-1216, 1.16% (male) and 2.73% (female) at 0.5 mg/kg and 2.17% (male) and 1.76% (female) at 50 mg/kg of the radio-labeled dose was eliminated in the urine, and 92.75% (male) and 93.88% (female) at 0.5 mg/kg and 86.48% (male) and 90.69% (female) at 50 mg/kg of the radio-labeled dose was eliminated in the feces 48 hours after dosing. For [pyridine- ^{14}C]-IKF-1216, 1.63% (male) and 2.93% (female) at 0.5 mg/kg and 2.94% (male) and 2.67% (female) at 50 mg/kg of radio-labeled dose was eliminated in the urine and 94.37% (male) and 98.43% (female) at 0.5 mg/kg and 56.98% (male) and 79.15% (female) at 50 mg/kg of the radio-labeled dose was eliminated in the feces 48 hours after dosing. Individual fecal samples were extracted with ether, acetonitrile, and water, and the extracts analyzed by HPLC. HPLC data indicate that the profiles of radioactivity extracted from feces are the same for the 2 label positions. **Supplemental study** because 1) 3 males and 3 females per dose were used rather than 4 males; 2) although between 56.98 and 98.43% of the labeled test articles were found in the feces, neither intravenous administration of the test articles and measurement of radioactivity in the excreta nor measurement of radioactivity in the bile was conducted; 3) no identification and quantitation of the metabolites of the test articles were conducted; 4) with the exception of the gastrointestinal tract, no other tissues were collected and frozen at termination. (Corlett and Leung, 01/20/2011)

0053; 246037; "Study to Measure the Pharmacokinetics of [Phenyl- ^{14}C]-IKF-1216 in the Blood of Rats" (Andre, J.C. et al., Ricerca, Inc., Department of Toxicology and Animal Metabolism, Painesville, OH, Study number 92-0262, 07/28/1994). 870.7485. IKF-1216, Lot #0201, 99.9% and [phenyl- ^{14}C]-IKF-1216, Ref. 91-67, purity= 99.5%, specific activity 2.109 Gbq/mmol. Suspensions of the test article in 0.75% (w/v) methylcellulose were administered as a single dose by gavage to 5 Sprague-Dawley Crl:CDBR VAF/Plus rats per sex per dose at dose levels of 0.5 and 50 mg/kg. In the case of the 50 mg/kg dose, a homogeneous mixture of labeled and non-labeled IKF-1216 was prepared, while, in the case of the 0.5 mg/kg dose, only the labeled compound was used. Each animal received between 47.73 and 75.95 uCi/kg of radioactivity. Two animals of each sex were dosed with the vehicle only. Blood was collected by orbital sinus puncture at 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 10, 12, 14, 18, 24, 48, and 72 hours and all samples were analyzed for radiolabel. The median peak time for both sexes and both dose levels combined was 6 hours (with a range of 2 to 8 hours for the low dose and of 4 to 10 hours for the high dose). Average C_{max} = 32 ng-Equiv/g for low dose males, 39 ng-Equiv/g for low dose females, and 2900 ng-Equiv/g for high dose males and females. Median $T_{1/2}$ = 5.4 hours and 42 hours (low dose males, alpha-phase and beta-phase, respectively), 4.5 hours and 39 hours (low dose females, alpha-phase and beta-phase, respectively), 4.9 hours (combined low dose male and females, alpha-phase), 32 hours (high dose males), and 27 hours (high dose females). Median AUC= 900, 1200, and 1000 ng-equiv. x hour/g for low dose male, female, and combined male/female, respectively, and 96,200, 104,800, and 100,400 ng-equiv. x hour/g for high dose male, female, and combined male/female, respectively. **Supplemental study** (this is a study to determine the time course concentration of radioactivity in the blood of rats; the concentration of the radioactivity in the expired air, urine, feces, and tissues of the treated rats was not determined). (Corlett and Leung, 01/26/2011)

0054; 246038; "Study to Evaluate the Distribution and Excretion of [Phenyl-¹⁴C]-IKF-1216 (¹⁴C(B)-IKF-1216) in Rats" (Andre, J.C. et al., Ricerca, Inc., Department of Toxicology and Animal Metabolism, Painesville, OH, Study identification 92-0185, 12/12/1994). 870.7485. The test article, [phenyl-¹⁴C]-IKF-1216, Ref. 91-67, purity= 99.5%, specific activity 2.109 Gbq/mmol was suspended in 0.75% (w/v) methylcellulose and administered as a single dose by gavage to 5 Sprague-Dawley Crl:CDBR VAF/Plus rats per sex per dose at dose levels of 0.5 and 50 mg/kg. In the case of the 50 mg/kg dose, a homogeneous mixture of labeled and non-labeled (IKF-1216, Lot #0201, purity = 99.9%) IKF-1216 was prepared, while in the case of the 0.5 mg/kg dose, only the labeled compound was used. Each animal received between 55.21 and 69.18 uCi/kg of radioactivity. 2 animals per sex were dosed with the vehicle only. Feces and urine were collected for 168 hours after the administration of the dosing material. Organs, blood, and cage washes were collected at termination. 2.16% (male) and 4.32% (female) at 0.5 mg/kg and 3.97% (male) and 3.96% (female) at 50 mg/kg of the radio-labeled dose (mean values) were eliminated in the urine, 93.90% (male) and 88.78% (female) at 0.5 mg/kg and 94.23% (male) and 91.60% (female) at 50 mg/kg of the radio-labeled dose (means values) were eliminated in the feces 168 hours after dosing. 0.55% (male) and 0.59% (female) at 0.5 mg/kg and 0.50% (male) and 0.47% (female) at 50 mg/kg (means values) of the radio-labeled dose were found in the tissues and carcass of the animals. **Supplemental study** since no measurement of radioactivity in the bile was conducted and no identification and quantitation of the metabolites of the test article were conducted. (Corlett and Leung, 01/31/2011)

0055; 246039; "Study to Evaluate the Distribution and Excretion of [Phenyl-¹⁴C]-IKF-1216 in Rats Following Repeated Dosing" (Andre, J.C. et al., Ricerca, Inc., Department of Toxicology and Animal Metabolism, Painesville, OH, Study No. 93-0021, 11/02/1994). 870.7485. Non-labeled IKF-1216 (lot no. 0301, purity= 99.9%) was suspended in 0.75% (w/v) methylcellulose and administered as a single dose by gavage at 24-hour intervals during a 14-day period to 2 sets of 5 Sprague-Dawley Crl:CDBR VAF/Plus rats per sex at dose level of 0.5 mg/kg. After this 14-day dosing period both sets of rats were dosed once by gavage with [Phenyl-¹⁴C]-IKF-1216 (Ref. 91-67, purity= 99.5%, specific activity 2.109 Gbq/mmol) suspended in 0.75% (w/v) methylcellulose. Each animal received between 8.83 and 16.70 uCi/animal of radioactivity. Urine and feces were collected until termination of the animals. 5 animals per sex were terminated 24 hours after [Phenyl-¹⁴C]-IKF-1216 dosing and the remaining 5 animals per sex were terminated 168 hours after [Phenyl-¹⁴C]-IKF-1216 dosing. 2 animals per sex were dosed with the vehicle only. 1.16% (males) and 2.84% (females) of the radio-labeled dose (mean values) were eliminated in the urine in animals sacrificed 24 hours after dosing with labeled test article while 1.36% (males) and 3.52% (females) of the radio-labeled dose (mean values) were eliminated in the urine in animals sacrificed 7 days after dosing with labeled test article. 91.73% (males) and 83.83% (females) of the radio-labeled dose (mean values) were eliminated in the feces in animals sacrificed 24 hours after dosing with labeled test article while 93.49% (males) and 100.03% (females) of the radio-labeled dose (mean values) were eliminated in the feces in animals sacrificed 7 days after dosing with labeled test article. 8.61% (males) and 11.81% (females) of the radio-labeled dose (mean values) were found in the tissues of animals sacrificed 24 hours after dosing with labeled test article while 0.55% (males) and 0.55% (females) of the radio-labeled dose (mean values) were found in the tissues of animals sacrificed 7 days after dosing with labeled test article. 3.95% (males) and 7.55% (females) of the radio-labeled dose (mean values) were found in the gastrointestinal tract of animals sacrificed 24 hours after dosing with labeled test article while 0.06% (males) and 0.07% (females) of the radio-labeled dose (mean values) were found in the gastrointestinal tract of animals sacrificed 7 days after dosing with labeled test article. **Supplemental study** since no measurement of radioactivity in the bile was conducted and no identification and quantitation of the metabolites of the test article were conducted. (Corlett and Leung, 02/02/2011)

0056; 246040; "Study of the Biliary Excretion of Radiolabel Following Oral Administration [Phenyl-¹⁴C]-IKF-1216 to Male Sprague-Dawley Rats" (Marciniszyn, J.P. et al., Ricerca, Inc.,

Department of Toxicology and Animal Metabolism, Painesville, OH, Study No. 92-0321, 02/06/1995). 870.7485. The test article, [phenyl-¹⁴C]-IKF-1216, Lot No. 91-67, purity > 98%, specific activity 2.109 Gbq/mmol was suspended in aqueous 0.75% (w/v) methylcellulose and administered as a single dose by gavage to male Sprague-Dawley Crl:CDBR VAF/Plus rats, 7 dosed with 0.5 mg/kg and 6 dosed with 50 mg/kg. In the case of the 50 mg/kg dose, a homogeneous mixture of labeled and non-labeled (IKF-1216, Lot no. 0201, purity = 99.9%) IKF-1216 was prepared, while in the case of the 0.5 mg/kg dose, only the labeled compound was used. 2 males were dosed with the vehicle only. Each animal at the 0.5 mg/kg dose level received between 57.1 and 58.9 uCi/kg of radioactivity and at the 50 mg/kg dose level received between 190.0 and 203.0 uCi/kg. Bile, feces, and urine were collected from all animals for 48 hours after the administration of the dosing material. The following mean percentages of the radio-labeled dose were collected by 48 hours- 0.5 mg/kg dose: 33.90 (bile), 2.23 (urine), 2.40 (carcass), 0.14 (blood), 0.18 (cage wash), 38.86 (absorbed), 48.44 (feces), 3.74 (gastrointestinal tract), and 90.04 (total recovery); 50 mg/kg dose: 25.03 (bile), 1.21 (urine), 2.35 (carcass), 0.10 (blood), 0.20 (cage wash), 28.89 (absorbed), 61.51 (feces), 2.98 (gastrointestinal tract), and 93.38 (total recovery). **Supplemental study** since the liver, spleen, fat, and kidneys were not collected and the amounts of radioactivity in these organs were not determined; also, the results from the isolation and identification of metabolites of the test article in the bile were not presented in the test report. (Corlett and Leung, 02/08/2011)

0057, 246041:[Addendum to Document #'s (Record #'s) 51977-0054 (246038), -0055 (246039), -0056 (246040)] "Study to Identify the Metabolites of IKF-1216 (Fluazinan) in Rats : I" (McClanahan, R.H., Department of Environmental and Metabolic Fate, Ricerca, Inc., Painesville, OH, Project Identification 92-0191, 12/20/1994 (interim report)). Metabolites were identified from samples of feces, urine, and bile obtained from animals in the following studies: Study to Evaluate the Distribution and Excretion of [Phenyl-¹⁴C]-IKF-1216 (¹⁴C(B)-IKF-1216) in Rats (Record # 246038), Study to Evaluate the Distribution and Excretion of [Phenyl-¹⁴C]-IKF-1216 in Rats Following Repeated Dosing (Record #246039), and Study of the Biliary Excretion of Radiolabel Following Oral Administration [Phenyl-¹⁴C]-IKF-1216 to Male Sprague-Dawley Rats (Record #246040). Techniques used to identify the metabolites included HPLC coelution with standards, direct identification by mass spectrometry and identification with standards, NMR of metabolites and comparison with standards, and base hydrolysis. In the 50 mg/kg dose group of the distribution study, the majority (from 60 to 99%) of the radioactivity was in the pooled feces and the major components of the extractable organic fraction of the pooled feces were unmetabolized IKF-1216, the metabolite AMPA, and the metabolite DAPA totaling 53.1% (in males) and 68.2% (in females) of the administered dose. In the 0.5 mg/kg dose group of the distribution study, the majority (from 50 to 67%) of the radioactivity was in the pooled feces and the major components of the extractable organic fraction of the pooled feces were unmetabolized IKF-1216, AMPA, and DAPA totaling 15.5% (in males) and 11.2% (in females) of the administered dose. There were several minor metabolites in the aqueous fraction of the fecal extracts but these were not characterized. In the pooled feces, unmetabolized IKF-1216, AMPA, and DAPA accounted for 33.7% (in males) and 41.3% of the administered dose in the repeated dosing study with the pattern of metabolite distribution in the extractable organic and aqueous fractions similar to those in the 50 mg/kg group of the distribution study. Urine contained less than 5% of the administered dose for both the distribution and repeated dosing studies; AMPA mercapturate and DAPA were identified as metabolites in the urine. AMPA mercapturate and DAPA glucuronide were identified as major metabolites in the bile (from the biliary excretion study); because of low levels, minor metabolites were not characterized. Data presented indicate that IKF-1216 was metabolized by both reduction and by glutathione conjugation and further metabolism. **Supplemental study** (only metabolite identification was conducted). (Corlett and Leung, 02/10/2011)

0058, 246042:[Addendum to Document #'s (Record #'s) 51977-0054 (246038), -0055 (246039), -0056 (246040), -0057 (246041)] "Study to Identify the Metabolites of IKF-1216 (Fluazinan) in Rats: Final Report" (McClanahan, R.H., Department of Environmental and

Metabolic Fate, Ricerca, Inc., Painesville, OH, Study Number 92-0191, 09/15/1995). Metabolites were identified from samples of feces, urine, and bile obtained from animals in the following studies: Study to Evaluate the Distribution and Excretion of [Phenyl-¹⁴C]-IKF-1216 (¹⁴C(B)-IKF-1216) in Rats (Record # 246038), Study to Evaluate the Distribution and Excretion of [Phenyl-¹⁴C]-IKF-1216 in Rats Following Repeated Dosing (Record #246039), and Study of the Biliary Excretion of Radiolabel Following Oral Administration [Phenyl-¹⁴C]-IKF-1216 to Male Sprague-Dawley Rats (Record #246040). An additional biliary metabolism study was conducted as part of this study (Study Number 92-0191), a study comparing the metabolism of [phenyl-¹⁴C]-IKF-1216 and [pyridyl-¹⁴C]-IKF-1216 to determine if metabolic cleavage of the phenyl and pyridyl rings of IKF-1216 occurs. In this additional biliary study, test articles [phenyl-¹⁴C]-IKF-1216 (Lot: 93-5, radiochemical purity = 98%, specific activity 2.12 Gbq/mmol) and [pyridyl-¹⁴C]-IKF-1216 (Lot: 93-90, radiochemical purity = 98%, specific activity 2.45 Gbq/mmol) were suspended in aqueous 0.75% (w/v) methylcellulose and administered as a single dose by gavage to 3 Sprague-Dawley Crl:CDBR VAF/Plus rats per sex per dose per label at a dose level of 50 mg/kg (homogeneous mixtures of labeled and non-labeled (IKF-1216 (Lot no. 0301, purity = 99.9%) IKF-1216 were prepared). Techniques used to identify the metabolites included HPLC coelution with standards, direct identification by mass spectrometry and identification with standards, NMR of metabolites and comparison with standards, and base hydrolysis. Bile, feces, and urine were collected from all animals for 48 hours after the administration of the dosing material. Major metabolites isolated included IKF-1216, AMPA, DAPA, DAPA glucuronide, and AMPA mercapturate. In the additional biliary study, the identified metabolites were the same in samples from both phenyl and pyridyl labels indicating that metabolic cleavage of the two rings did not occur. Data presented in the additional biliary study and the other studies reviewed indicate that IKF-1216 was metabolized by both reduction and by glutathione conjugation and further metabolism. **Supplemental study** (individual animal data of the additional biliary study were not presented in the test report). (Corlett and Leung, 02/16/2011)